Amendments to the Specification

Referring to the published application no. 2002-0198372 for all amendments to the specification following.

Please replace paragraph [0010] with the following amended paragraph:

[0010] The present invention provides automatable methods for purifying plasmid DNA from cells. The methods involve use of a static mixer to mix the cells with a lysis solution to provide controlled, gentle mixing of the cells with the lysis solution. Static mixers are further used to mix the resulting lysis mixture with a precipitation solution to precipitate out cell debris and other contaminants, including chromosomal DNA. This is typically followed by an additional step of centrifugation to remove the precipitated material. The methods of the invention are sufficient to provide a purified DNA solution that does not require complex purification steps (e.g., ultrafiltration) prior to application to an ion exchange chromatography to produce a final product.

Please replace paragraph [0033] with the following amended paragraph:

[0033] The lysis mixture exiting the static mixer **30** then flows through line **60** to static mixer **70**. A tank **80** containing a precipitating solution is connected through line **90** to line **60**. The precipitating solution is used to precipitate proteins, chromosomal DNA and cell debris. Typically, the solution will contain potassium acetate. A suitable precipitating solution is 3M potassium acetate, adjusted to pH 5.5, with acetic acid (~5M acetate final). As with the first static mixer, the intersection between lines **60** and **90** can be adjusted so that the lysis mixture and the precipitation solution enter the static mixer [[**30**]] **70** essentially simultaneously. Similar linear velocities are used to ensure sufficient mixing to thoroughly precipitate the proteins and cellular debris and yet not be so high that genomic DNA is sheared to a size that is problematic in later purification steps. Typically, approximately 5 liters of precipitating solution is used per kg of cell paste.

Please replace paragraph [0034] with the following amended paragraph:

[0034] After exiting static mixer [[**30**]] **70**, the precipitating solution flows through line 100 to centrifuge 110. In some embodiments, a tank 120 containing a buffer solution is connected to line 100 through line 130. The buffer solution is used to raise the pH of the solution to minimize acid catalyzed de-purination of the DNA and to condition the material for binding onto the anion exchange column, e.g., a pH is the range of 6 to 9, preferably from 7 to 8.5. A useful buffer solution for this purpose is 1 M Tris. More concentrated solutions can be used to adjust the pH, however the use of a more diluted buffer solution has other benefits. A diluted buffer reduces the viscosity of the solution going into the centrifuge to yield better clarity. It also decreases the ionic strength of the solution, such that it can be loaded directly onto the anion exchange column.

Please replace paragraph [0040] with the following amended paragraph:

[0040] A chromatography column is packed with an anion exchange chromatography resin. The optimal capacity of the column is determined empirically based on the resin used and the size of the nucleic acid to be purified. The column is packed under low pressure, typically less than about [[7]] 0.7 bar. The pressure will depend on the resin used, and will usually be according to the manufacturer's specifications. Normal column operating pressure may be lower where the resin pore size is smaller, to limit trapping of the nucleic acid in the resin pores. Thus, for resins without pores, column operating pressure may be increased. The column is packed at about twice the anticipated flow rate in accordance with conventional techniques.

Please replace paragraph [0064] with the following amended paragraph:

[0064] The cell pellets were spread into thin sheets and frozen at -80° C. until used for further plasmid purification. 3.2 Kg of the cell pellet was resuspended in 16L Solution I (25 mM Tris-HCl, pH 8, 10 mM EDTA, 50 mM dextrose) at room temperature with stirring at 150 rpm for 1 h. RNase digestion was achieved by the addition of RNase (305 mg RNase/Kg cell paste) and incubating the solution on ice for 2 hrs. Cells were lysed by the addition of the cells to 32L Solution II (0.2N NaOH/1%SDS) in an ice bath. The solution is stirred using a Bow-Tie Stirrer (Cole Parmer, Vernon Hills, Ill.) for 25 min. This solution was then neutralized and cell debris and chromosomal DNA were precipitated by the addition of 16L ice-cold Solution III (3M potassium, [[SM]] 5M acetate, pH 5.5). The solution was mixed with a Bow-Tie Stirrer on ice for 25 min.

Please replace paragraph [0083] with the following amended paragraph:

[0083] Approximately 40 L of fermentation broth yields about 2.2 kg of cell paste. After re-suspension of the cell paste, lysis and precipitation, approximately 40 liters of solution were ready for clarification by centrifugation. Centrifuging in a non-continuous centrifuge (Sorvall RC3b) at 7500 x g for 25 minutes removed the solids and yielded a clarified product. Tris base (solid) was added to adjust the pH of the clarified product to 8.5 (a final concentration of 0.67 M). After Tris base addition, the conductivity decreased from 53 mS/cm to 50 mS/cm. The neutralized lysate was filtered in series with a nominal 0.2 µm glass filter (Sartorpure GF) and an absolute [[0.2 m]] 0.2 µm nylon filter (Pall Ultipor N_{66}) (5 ft^2 each) to reduce bacterial load and endotoxin levels.

Please replace paragraph [0085] with the following amended paragraph:

[0085] The pooled product contained 1787 mg DNA, endotoxin level of 16 EU/mg, and 1.6% genomic DNA. The product was filtered again through [[0.2 m]] 0.2 µm nominal glass and [[0.2 m]] 0.2 µm absolute nylon filters described above. After filtering the product contained endotoxin level of 1 EU/mg and 0.18% genomic DNA, with 94% yield. The filtered product was diafiltered and subjected to a final [[0.2 m]] 0.2 µm sterilization filter as described in Example 1. The final product was 5.6 mg/ml, with 0.4 EU/mg and less than 0.2% genomic DNA.